ELSEVIER

Aquatic Botany 50 (1995) 193–200

Aquatic botany

Effects of low temperature and physiological age on superoxide dismutase in water hyacinth (*Eichhornia crassipes* Solms)

Xuebao Li[a,*], Zhenbin Wu[b], Guangyuan He[c]

[a]*Department of Biology, Normal University of Central China, Wuhan 430070, China*
[b]*Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, China*
[c]*Wuhan Institute of Botany, The Chinese Academy of Sciences, Wuhan 430074, China*

Accepted 29 June 1994

Abstract

Superoxide dismutase activity in water hyacinth leaves was not sensitive to small changes in environmental pH, but declined markedly with greater pH changes. KCN inhibited superoxide dismutase activity, suggesting that the enzyme was mainly composed of the Cu–Zn form. Low temperature (2°C) treatment caused a decline in superoxide dismutase activity. This effect became more pronounced as the treatment time was prolonged. Furthermore, the decline was much more significant than reductions of glucose-6-phosphate dehydrogenase activity or respiration under comparable conditions. With increasing physiological age, superoxide dismutase activity declined and was significantly lower in old than in young leaves. Therefore, superoxide dismutase activity might be employed as one of physiological parameters in studying leaf senescence.

1. Introduction

The water hyacinth (*Eichhornia crassipes* Solms) is one of the most productive plants in the world. Over the last two decades, it has been widely used to treat domestic and some industrial wastewaters in China (Wu et al., 1987, 1988, 1990). In temperate areas, the water hyacinth treatment systems generally give good purification efficiencies. However, in cold weather in most cool temperate zones

* Corresponding author.

0304-3770/95/$09.50
SSDI 0304-3770(94)00417-K

growth is poor (Wu et al., 1987). The physiological mechanism of the tolerance of water hyacinth to low temperature is worthy of study.

Superoxide dismutase (EC 1.15.1.1), a kind of scavenger which removes superoxide radicals, is involved in the minimisation of radical damage and prevention of senescence in plants (Wang et al., 1989). It was reported that the enzyme was related to plant resistance to pollution, dryness and cold (Wang et al., 1989). We have investigated the activity of this enzyme in relation to the developmental stage of water hyacinths, different environmental pH and low temperature.

2. Materials and methods

Water hyacinths collected from East Lake, Wuhan, were grown and propagated in pools in the greenhouse (25–30°C, sunlight). Some plants were sampled and cultured in Hoagland nutrient culture solution as experimental materials. Leaf development was observed daily. When leaf primordia were detectable by the naked eye, their physiological age was recorded. The 3-day-old leaves were greenish and the leaf cells divided rapidly, so the cells were small. As the leaves developed further, they became dark green in colour and the cells enlarged in size and developed vacuoles. The leaves reached maturity in about 9–12 days, by which time the leaf vascular system had become established, and cell division ceased. After 30–35 days, the leaves became yellow-green and large vacuoles formed in the cells, indicative of leaf senescence. After being washed with tap water, the plants were transferred to and cultured in fresh nutrient solution at 2°C for 4, 8, 12, 16 and 24 h; control plants were kept at 28 ± 1°C. The experiments were conducted in darkness. Leaves of different developmental stages were collected simultaneously from treatment and control plants for the measurement of age and physiological parameters.

After the leaves were surface-sterilised and washed with sterile water five to seven times, 2 g (fresh weight) of water hyacinth leaves were placed in a glass homogeniser on ice with 5 ml of 0.02 mol l^{-1} sodium phosphate buffer (pH 7.8), homogenised thoroughly on ice and then filtered through a two-layer gauze. In experiments on the effect of pH, buffers of different pH were used. Filtered liquid was centrifuged at 13 000 *g* for 20 min at 4°C. Pellets were discarded, and supernatant was collected as crude superoxide dismutase extract for further experiments.

The assay of superoxide dismutase activity was performed according to Giannoplilis and Ries (1977). It was based on the inhibition of nitroblue tetrazolium (NBT) photochemical reduction by superoxide dismutase. The reaction mixture contained 0.013 mol l^{-1} methione, 1.3×10^{-6} mol l^{-1} riboflavin, 7.5×10^{-5} mol l^{-1} NBT, and 0.05 mol l^{-1} phosphate buffer (pH 7.8); 50 μl of crude enzyme extract was added to a final 3 ml of reaction mixture. The reaction was initiated and lasted 15 min (37°C) under 4000 lx fluorescence light. The optical density

at 560 nm was recorded. In controls the crude enzyme extract was omitted and replaced by phosphate buffer. Superoxide dismutase activity was defined as percentage inhibition of NBT.

Protein contents were determined according to Lowry et al. (1951). The measurement of leaf respiratory rates was made by oxygen electrode (Li and Ye, 1980). Glucose-6-phosphate dehydrogenase activity was determined according to Ouyang (1985).All experiments were replicated eight times.

3. Results

3.1. Effects of KCN and pH on superoxide dismutase

NBT photochemical reduction was linear for at least 20 min after initiation of the reaction (Fig. 1) and was clearly inhibited by water hyacinth leaf superoxide dismutase. The percentage of inhibition was correlated with the amount of crude enzyme extract added (Fig. 2).

After the addition of a certain amount of potassium cyanide (KCN) the inhibition became weaker, indicating decreased enzyme activity. The degree of decrease in enzyme activity was nearly proportional to KCN concentration. At a KCN concentration of 0.3×10^{-3} mol l^{-1}, enzyme activity decreased by 90% (Table 1). Superoxide dismutase activity differed with pH, being almost unaltered under weak acid and/or weak alkaline conditions, but markedly reduced in both strongly acidic (pH 3) and strongly alkaline (pH 12) conditions (Fig. 3).

3.2. Effect of low temperature on superoxide dismutase

When water hyacinths were incubated at 2°C for 4 and 8 h, superoxide dismutase activity was almost unaffected, but declined progressively faster in the 12, 16 and 24 h treatment groups (Table 2). Analysis of variance showed that differences between the treatment groups and control group were significant.

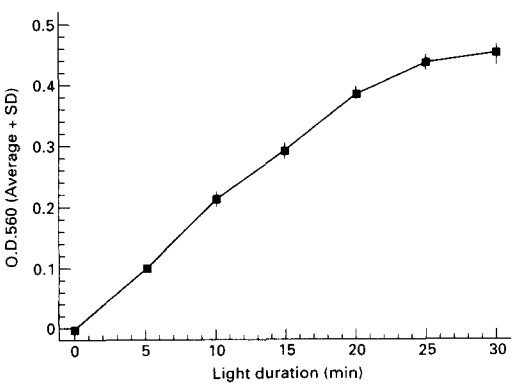

Fig. 1. NBT photochemical reduction rate during a 30 min period.

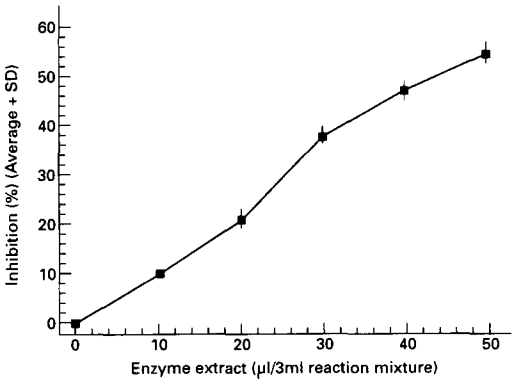

Fig. 2. Inhibition of NBT photochemical reduction by the addition of crude superoxide dismutase extract of water hyacinth leaves.

Table 1
Effect of KCN on superoxide dismutase (SOD) activity

KCN conc. ($\times 10^{-3}$mol l^{-1})	Replicates	Average SOD activity (μ mg^{-1} protein)	Standard error (interval)
0	8	178.2	3.12
0.05	8	140.4	2.76
0.1	8	116.8	2.22
0.2	8	70.3	1.79
0.3	8	15.6	0.56
		$P<0.01$	

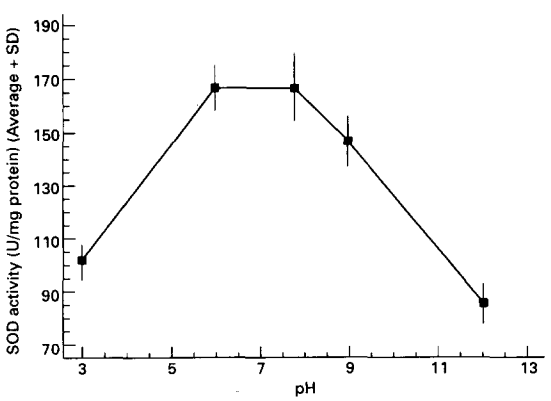

Fig. 3. Effect of pH of extracting buffer on superoxide dismutase activity in water hyacinth leaf.

Table 2
Effects of low temperature (2°C) on mean (±SD) superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase activities and respiration in water hyacinth leaf

Treatment time (h)	SOD activity (μ mg^{-1} protein)	G-6-p dehydrogenase activity (SOD mg^{-1} protein min^{-1})	Respiration (μl O g^{-1} min^{-1})
0	170.0±2.17 a	0.098+0.0015 a	3.27+0.0204 a
4	168.7±1.86 b	0.095+0.0021 b	3.18+0.0096 b
8	164.0±1.19 b	0.095+0.0011 bc	3,29+0.0241 c
12	118.8±2.20 c	0.092+0.0021 c	3.11+0.0296 cd
16	118.0±1.47 c	0.087+0.0013 c	3.01+0.0335 de
24	83.0±0.49 c	0.055+0.0014 c	2.86+0.0238 e
	$P<0.01$	$P<0.01$	$P<0.01$

Letters denote interval homogeneous groups.

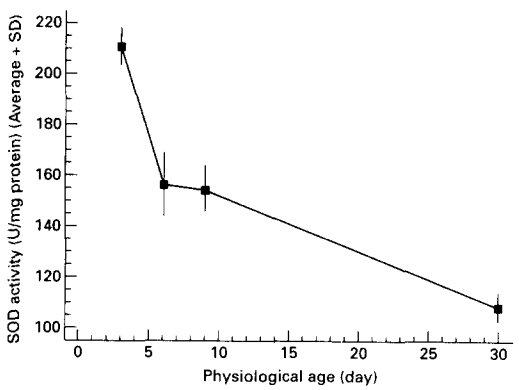

Fig. 4. Activity of superoxide dismutase of water hyacinth leaf at different leaf developmental stages.

3.3. Change of superoxide dismutase activity of leaves at different physiological ages

Leaves 3, 6, 9 and 30 days of age were analysed for superoxide dismutase activity. The results of eight replicate experiments showed that enzyme activity was highest in 3-day-old leaves, followed by 6- and 9-day-old leaves, and was lowest in 30-day-old leaves (Fig. 4). The differences in enzyme activity were highly significant between young and senescent leaves.

3.4. Effect of low temperature on respiration and glucose-6-phosphate dehydrogenase activity

Within a short period of time (8 h) of low temperature treatment, a slight change was found in the respiratory rates of water hyacinth leaves. After 16 h of

treatment, however, both respiratory rate and glucose-6-phosphate dehydrogenase activity decreased. After 24 h, leaf respiration slowed markedly and the activity of glucose-6-phosphate dehydrogenase, a key enzyme in cell metabolism and respiration, also decreased (Table 2). This implied that leaf metabolism was affected and seriously damaged by low temperature.

4. Discussion

Superoxide dismutase occurs in three forms, the Cu–Zn type, the Mn type and the Fe type. The Cu–Zn type is universally present in animals and plants. Cu–Zn superoxide dismutase activity was not sensitive to small changes in pH, but declined with greater pH changes, and was sensitive to KCN. In our experiment, the activity of this enzyme was only slightly affected by pH alteration, but was suppressed by KCN (Table 1), indicating that in water hyacinth leaves the enzyme mainly consisted of the Cu–Zn type.

Under low temperature conditions, superoxide dismutase activity in plants declined. Other studies have shown that after being stored in the dark at 0°C for 3 days, most of the enzyme activity in tomato leaves was lost (Michalski and Kaniuga, 1981). At 5°C the enzyme activity in rice leaves decreased by 10–30% (Wang et al., 1986). Low temperature caused the enzyme to lose 11.57–26.5% of its activity in cucumber (Liu et al., 1985). Our experiment also revealed that enzyme activity in hyacinth leaves was strongly affected by low temperature and its decrease was dependent on the duration of low temperature. Some enzymes in plants are highly sensitive to low temperature. Chilling stress caused a decrease in enzyme activity or even loss of functions and resulted in the accumulation of some toxic substances, cell metabolic disturbance and destruction of membrane systems (Wade, 1979; Omran, 1980; Lyons, 1983; Wise and Naylor, 1987; Yang and Liu, 1988). Superoxide dismutase plays an important role in the protection of membrane lipid from peroxidation (Wang et al., 1989). In our experiment, the decrease in enzyme activity was characteristic of enzymes which are highly sensitive to low temperature stress. Plant resistance to low temperature might be more related to superoxide dismutase activity than to other physiological factors, suggesting that elevating enzyme activity should be a priority if we intend to improve the resistance of the water hyacinth to low temperature.

Within 8 h of low temperature superoxide dismutase activity remained almost unchanged, but the longer the treatment lasted, the more obvious was the decline in enzyme activity (Table 2). It is worthwhile mentioning that superoxide dismutase activity was able to remain almost unchanged within a certain period of time (8 h) at low temperature. This might offer an opportunity to improve the resistance of the plant to chilling stress through cold acclimation and other biochemical or/and biological approaches. With leaf senescence, superoxide dismutase and catalase activities decreased. At the same time, cell membrane permeability and lipid peroxidation were enhanced and free radicals accumulated in leaves (Dhindsa et al., 1981; Wang et al., 1989). Our experimental re-

sults also showed that the decrease in superoxide dismutase activity paralleled leaf senescence in water hyacinth. Therefore, this enzyme may be employed as a physiological parameter in studying leaf senescence in water hyacinth.

Acknowledgement

The project was supported by the International Foundation for Science (IFS, G/1579-1). The authors wish to express their thanks to IFS for support, advice and encouragement. Special thanks to Professor Wang Deming, Professor Ley Shanghao, Dai Heping and Dr. Cui Yibo for their help and valuable comments on the manuscript.

References

Dhindsa, R.S., Plumb-Dhindsa, P. and Thorpe, T.A., 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32:93–101.

Giannoplilis, C.N. and Ries, S.K., 1977. Superoxide dismutase II. Purification and quantitative relationship with water-soluble protein in seedlings. Plant Physiol., 59:315–318.

Li. D.Y. and Ye, J.W., 1980. Preparation of oxygen electrode with polyethylene membrane and its application in the measurement of respiration or photosynthesis control. Plant Physiol. Commun., (1): 35–40. (In Chinese.)

Liu, H.X, Zeng, S.X., Wang, Y.R., Li, P., Chen, D.F. and Guo, J.Y., 1985. The effect of low temperature on superoxide dismutase in various organelles of cucumber seedling cotyledon with different cold tolerance. Acta Phytophysiol. Sinica, 11(1):48–57. (In Chinese with English abstract.)

Lowry, O.H., Rosebrough, N.J., Faar, A.L. and Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193:265–275.

Lyons, J.M., 1983. Chilling injury in plants. Annu. Rev. Plant Physiol., 24:445–466.

Michalski, W.P. and Kaniuga, Z., 1981. Photosynthetic apparatus of chilling- sensitive plants. X. Relationship between superoxide dismutase activity and photoperoxidation of chloroplast lipids. Biochem. Biophys. Acta, 637:195–167.

Omran, R.G., 1980. Peroxide level and the activity of catalase, peroxidase and indoleacetic acid oxidase during and after chilling cucumber seedling. Plant Physiol., 65:407–408.

Ouyang, G.C., 1985. In: Y. Xue and Z. Xia (Editors), Handbook of Plant Physiology Experiments. Shanghai Science and Technology Press, Shanghai, pp. 183–184. (In Chinese.)

Wade, N.L., 1979. Physiology of cool storage disorders of fruit and vegetable. In: J.M. Lyons, D. Graham and J.K. Raison (Editors), Low Temperature Stress in Crop Plants: The Role of the Membrane. Academic Press, New York, pp. 82–96.

Wang, J.H., Liu, H.X. and Xu, T., 1989. The role of superoxide dismutase in stress physiology and senescence physiology of plant. Plant Physiol. Commun., (1):1–7. (In Chinese.)

Wang, Y.R., Liu, H.X., Li, P., Zeng, S.X., Zhen, L.P. and Guo, J.Y., 1986. The effect of chilling stress on membrane-lipid peroxidation of photosynthetic apparatus in rice seedlings in the dark and light. Acta Phytophysiol. Sinica, 12(3):244–251. (In Chinese, with English abstract.)

Wise, R.R. and Naylor, A.W., 1987. Chilling-enhanced photo oxidation: The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. Plant Physiol., 83:272–275.

Wu, Z.B., Qiu, C., Xia, Y.Z. and Wang, D.M., 1987. Studies on the purification of the Yanshan petrochemical wastewater by means of water hyacinth I. Dynamic simulation test. Acta Hydrobiol. Sinica, II(2):139–150. (In Chinese, with English abstract.)

Wu, Z.B., Xia, Y.Z., Qiu, C.Q. and Wang, D.M., 1988. Effects of the phenol in petrochemical wastewater on the growth of water hyacinth. Acta Hydrobiol. Sinica, 12(2):125–132. (In Chinese with English abstract.)

Wu, Z.B., Qiu, C.Q., Xia, Y.Z. and Wang, D.M., 1990. Effects of the salinity in petrochemical wastewater on the growth and purification efficiency of water hyacinths. Acta Hydrobiol. Sinica, 14(3):239–246. (In Chinese, with English abstract.)

Yang, X.Y. and Liu, C.D., 1988. Relationship between chilling injury and adenylate metabolism in rice seedlings. Acta Phytophysiol. Sinica, 14:344–349. (In Chinese with English abstract.)